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Influence of sulfoxide group placement on polypeptide conformational stability

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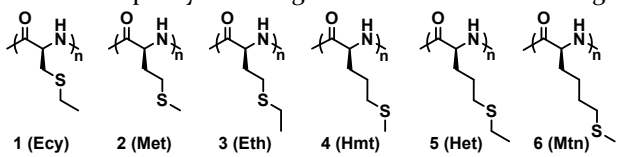
ABSTRACT: The synthesis of a homologous series containing five new non-ionic sulfoxide containing polypeptides was described. Sulfoxide groups bestowed water solubility for all homologs, which allowed their use as a model for study of helix-coil transitions in water while avoiding contributions from charged groups or phase separation. Polypeptides were found to adopt chain conformations in water that were dependent on distance of sulfoxides from chain backbones, overall side-chain lengths, and solvent. These results allow preparation of polypeptide segments with different chain conformations without changing chemical functionality for potential use in structural studies and functional applications.

As a starting point toward the understanding of protein folding, the helix-coil transition has been the subject of intensive study for many decades.[1] Synthetic polypeptides have often been used as model systems for study of helix-coil transitions due to their compositional simplicity.[2] Much attention has been focused on non-ionic, water soluble homopolypeptides,[3] which avoid contributions from charged groups,[4] to investigate effects of side-chain length and hydrophobicity on α-helical stability in aqueous media. However, studies on side-chain elongation in these systems have been confounded by simultaneous conformation changes and loss of water solubility.[5] Poly(L-methionine sulfoxide) is non-ionic and water soluble due to polar sulfoxide groups, yet these chains adopt disordered conformations.[3,6] To study the influence of sulfoxides on chain conformations and solubility in greater detail, we prepared a homologous series of new sulfoxide containing polypeptides differing in side-chain length and sulfoxide placement. We found that sulfoxides bestowed good water solubility for all homologs, which in turn allowed determination of how precise placement of sulfoxide functionality affects α-helical stability in water. The results of these studies also allow preparation of non-ionic, water soluble polypeptide segments that can possess different chain conformations without altering their chemical functionality.

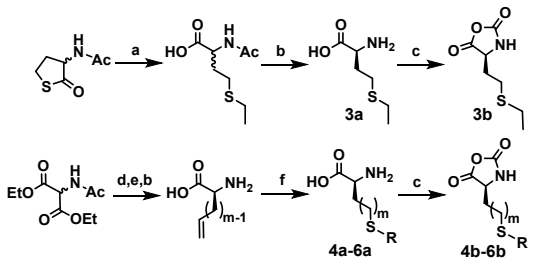
Many non-ionic, water soluble homopolypeptides are known, ranging from those that are α-helical[3,7] to those that are disordered in solution.[3,8] Studies on conformational effects due to side-chain placement of solubilizing groups have been limited to homologs of poly(L-serine)[4,9] and poly(hydroxyethyl-L-glutamine).[4,10] With the exception of water insoluble β-sheet forming poly(L-serine), stepwise elongation of side-chains for both of these polypeptides resulted in transitions from water soluble coils to water insoluble α-helices. The inability to decouple chain conformation from solubility, and consequent aggregation of α-helical chains, has limited the ability to study how the balance between side-chain hydrophobicity and chain solvation affect chain conformations in water.

In related studies, polypeptides containing oligoethylene glycol or saccharide functionality were found to undergo helix-coil transitions upon oxidation of thioethers to sulfoxides or sulfones.[3,11] Sulfoxides and sulfones are both significantly more polar than thioethers,[12] yet sulfoxides hydrogen bond well with water, while sulfones do not.[13] Consequently, sulfoxides generally provide excellent water solubility, while sulfones instead tend to self-associate.[14] In the polypeptides above, generation of sulfoxides primarily resulted in α-helix disruption, but outcomes varied depending on specific functionality present.[11] Based on our experience with poly(L-methionine sulfoxide), which has also been found to be non-fouling and possess good cell and tissue compatibility,[5,15] we chose to start with this polypeptide for a side-chain homologation study. Specifically, we sought to use this simple system to gain a better understanding of how side-chain placement of sulfoxides affect polypeptide conformations and solubility.



Scheme 1. Structures of homologous thioether containing polypeptides **1-6**.

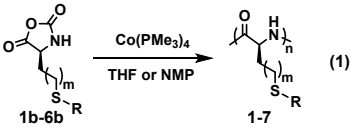
Poly(L-methionine), **2**, and the side-chain homologs used in this study are shown in Scheme 1. Two series were prepared, those containing terminal ethyl groups: S-ethyl-L-cysteine (Ecy), L-ethionine (Eth), and L-homoethionine (Het); and those containing terminal methyl groups: L-methionine (Met), L-homomethionine (Hmt), and 6-(methylthio)-L-norleucine (Mtn). Other than readily available Met, all other amino acids were synthesized using modifications of established procedures (Scheme 2, see Supporting Information (SI)). Ecy was prepared by direct alkylation of L-cysteine, and Eth was prepared by alkylation of DL-homocysteine followed by acylase mediated enantiomeric resolution. All other amino acids were prepared by synthesis of enantiomerically resolved alkene precursors, followed by thiol-ene addition of methanethiol (Scheme 2). The amino acids were subsequently converted to N-carboxyanhydride (NCA) monomers via phosgenation. All amino acids and NCAs were obtained with high optical purity in good yields at multigram scale.



Scheme 2. Synthesis of new thioether containing NCAs on gram scale. a) EtBr, K_2CO_3, H_2O; b) porcine acylase, H_2O, 40 °C; c) $COCl_2$, THF, 40 °C. d) Na°, allyl bromide or NaH, 3-butenyl bromide; e) NaOH, H_2O, 100 °C then pH 3.5, 100 °C; f) MeSH or EtSH, DMPA, *hv*. For Hmt (**4a-b**): m = 2, R = Me; Het (**5 a-b**): m=2, R = Et; Mtn (**6a-b**): m =3, R = Me.

The NCA monomers **2b-6b** were subsequently polymerized using $Co(PMe_3)_4$ initiator in THF at 20 °C (eq 1), where they remained soluble to degrees of polymerization (DP) of >100 (see SI Table S1).[16] Similar to previously reported **2b**,[13] new NCA monomers **3b-6b** underwent controlled polymerization with linear increase of chain length with monomer to initiator ratio, and were obtained in excellent yields and low dispersities ($Đ$ < 1.15) (Figure 1, Table 1, see SI Figure S1). Chain initiation efficiencies were consistent with prior results.[16] Polymerization of **1b** required use of more polar NMP solvent to minimize precipitation of the β-sheet forming polypeptide **1**. Polypeptides **2-6** were expected to adopt α-helical conformations based on their solubility in organic solvents and from studies of **2** and related polypeptides.[11,14] An example statistical copolymerization of **2b** and **6b** was found to give the copolypeptide **7** with expected composition (Table 1). Example block copolypeptides were prepared by sequential addition of NCA monomers (e.g. **5b** and **6b**) to initiator, and were obtained in high yields with expected compositions and low dispersities (Table 2).

Hydrophobic thioether containing polypeptides **1-7**, with designated average DP = 65 (Table 1), were subsequently oxidized using H_2O_2 to give the corresponding hydrophilic sulfoxide derivatives **1°-7°** in high yields (Figure S2). All sulfoxide derivatives **1°-7°** were found to readily dissolve in deionized (DI) water at 50 mg/mL, permitting study of chain conformations in the absence of chain aggregation. Solutions of each polypeptide sulfoxide in DI water at 22 °C were evaluated using circular dichroism (CD) spectroscopy to identify chain conformations. Polypeptide **1°** gave an unusual CD spectrum that most closely resembles a disordered conformation,[17] and was essentially unperturbed by solvent composition (Figure S3). It is likely that sulfoxide absorptions within the wavelengths studied,[18] combined with sulfoxide chirality and close proximity to the polypeptide backbone resulting in diastereomeric interactions, affected these CD spectra. Consequently, this sample was not included in further studies, and its properties will be investigated separately elsewhere.



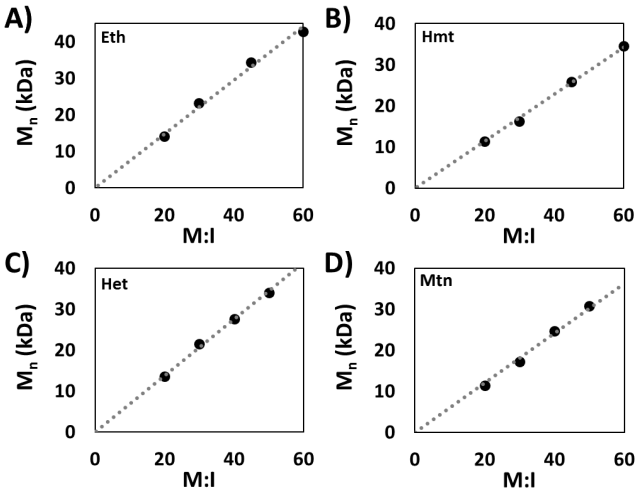


Figure 1. Molecular weights (M_n) as functions of monomer to initiator ratios (M:I) for NCA polymerizations using $Co(PMe_3)_4$ in THF at 20 °C. A) Eth NCA (**3b**); B) Hmt NCA (**4b**); C) Het NCA (**5b**); D) Mtn NCA (**6b**). Dotted lines show linear least squares fitting of data.

Table 1. Characterization data for thioether containing polypeptides.

Residue(s)	Sample	R	m	M_n[a] (kDa)	M_w/M_n[b]	DP[a]	Yield[e] (%)
Ecy	**1**	Et	0	8.8	--[c]	67	95
Met	**2**	Me	1	8.5	1.09	65	97
Eth	**3**	Et	1	9.0	1.13	62	99
Hmt	**4**	Me	2	9.0	1.09	62	98
Het	**5**	Et	2	10.4	1.09	65	99
Mtn	**6**	Me	3	10.5	1.14	66	100
Met/Mtn	**7**	Me	1,3[d]	9.0	1.10	62	97

[a] M_n and degree of polymerization (DP) determined by end-group analysis. [b] $Đ$ determined by GPC-MALS. [c] Sample aggregates in GPC solvent. [d] 1:1 statistical copolymer. [e] Total isolated yield of polymers. *R* and *m* as in equation 1.

Table 2. Synthesis of diblock copolypeptides using $Co(PMe_3)_4$ initiator in THF

First Segment			Second Segment		Diblock Copolypeptide[b]			
Monomer	M:I	DP[a]	Monomer	M:I	M_n[c] (kDa)	DP[c]	M_w/M_n[d]	Yield (%)[e]
Het NCA	20	60	Mtn NCA	60	38.2	240	1.09	97
Mtn NCA	20	61	Het NCA	60	38.9	244	1.10	95

[a] DP determined by endgroup analysis after polymerization of first segments. [b] Data for diblock copolypeptides after complete polymerization of second segments. [c] M_n and DP determined by end-group analysis. [d] $Đ$ determined by GPC-MALS. [e] Total isolated yield of diblock copolypeptides.

Polypeptides **2**^{O}-**7**^{O} gave more readily interpreted CD spectra showing varying degrees of disorder and α-helical content in DI water (Figures 2, 3A).[5] It was observed that increasing the side-chain alkyl spacers between polypeptide backbone and sulfoxides (i.e. **2**^{O} to **4**^{O} to **6**^{O}, and **3**^{O} to **5**^{O}) led to significant increases in α-helical content. Polypeptides **2**^{O} and **3**^{O} are disordered in DI water at 22 °C, while **5**^{O} and **6**^{O} predominantly adopt stable α-helical conformations. The *ca*. 58% α-helical content observed for **5**^{O} and **6**^{O} is significantly greater than the highest value observed (*ca*. 33%) in prior homologation studies.[10] These results showed that as polar, well-solvated sulfoxides are placed further from the backbone they are less able to perturb α-helical conformations and packing of hydrophobic alkyl side-chains. Notably, the equimolar statistical copolypeptide of Met and Mtn (**7**^{O}) was found to be highly disordered in water, similar to pure **2**^{O}, showing that a fraction of sulfoxides (i.e. 50%) closer to the backbone is also sufficient to disrupt formation of α-helical domains in water.

Polymer	R	m	Helicity (%)
2^{O}	Me	1	2
3^{O}	Et	1	3
4^{O}	Me	2	28
5^{O}	Et	2	58
6^{O}	Me	3	58
7^{O}	Me	1,3[a]	11

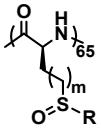


Figure 2. Conformations of sulfoxide containing polypeptides (0.5 mg/mL) in H_2O at 22 °C. α-Helix content determined from intensity of minima at 222 nm in CD spectra. a = 1:1 statistical copolymer.

Replacement of terminal methyls with ethyls had mixed effects on chain conformations. While **2**^{O} and **3**^{O} were both disordered in DI water, **5**^{O} possessed nearly twice the α-helical content compared to **4**^{O} (Figure 2). The latter comparison revealed a substantial conformational difference resulting solely from the change in terminal ethyl versus methyl groups. For **2**^{O} and **3**^{O}, the close proximity of sulfoxides to the backbone appears to overwhelm any α-helix stabilizing influence of the terminal alkyl groups. With increased sulfoxide distance from the backbone in **4**^{O} and **5**^{O}, their α-helix destabilizing influence was found to weaken relative to α-helix stabilizing effects from the terminal alkyl groups. Overall, we found that once sulfoxides are at least three C-C bonds removed from the backbone, non-ionic polypeptides can be prepared that are water soluble with high α-helical contents in solution. Further, initial studies on solutions of **5**^{O} and **6**^{O} at elevated temperature or in different ionic strength media showed that their α-helical conformations were minimally perturbed (Figures S4, S5).

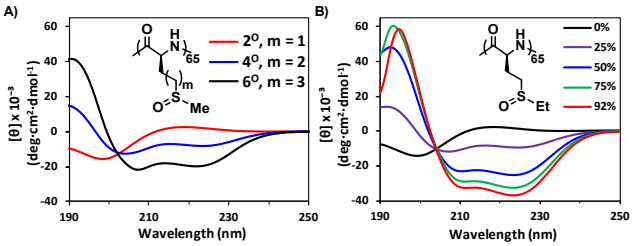


Figure 3. CD Spectra of select sulfoxide containing polypeptides (0.4-0.5 mg/mL) in H_2O or MeOH/H_2O mixtures at 22 °C. A) Variation of side-chain length using **2**^{O}, **4**^{O} and **6**^{O}. B) **3**^{O} in MeOH/H_2O mixtures (vol% methanol shown).

The results above suggest that close proximity of sulfoxides to polypeptide backbones disrupt α-helical conformations in water due to increased sulfoxide solvation near the backbone. An alternative hypothesis is that disorder arising from close proximity of sulfoxide groups of variable configuration to the backbone may instead be responsible for α-helix destabilization. It is known that peroxide mediated oxidation of Met leads to formation of sulfoxide groups with both possible configurations in roughly equal proportions.[19] In order to test whether solvation or stereochemical disorder of sulfoxides is primarily responsible for α-helix disruption, chain conformations of polypeptides **2**^{O}-**7**^{O} were evaluated in water/methanol mixtures. Methanol is known to promote formation of α-helical conformations due to its low relative permittivity compared to water, resulting in weaker H-bonding with polypeptide functionality.[20]

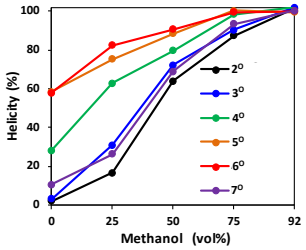


Figure 4. α-Helix content of sulfoxide containing polypeptides (0.4-0.5 mg/mL) in MeOH/H_2O mixtures at 22 °C.

Solutions of polypeptides **2**^{O}-**7**^{O} were prepared in media consisting of 0 to 92 v/v % methanol in water and evaluated using CD spectroscopy (Figures 3B, 4). For all samples, α-helical content was found to increase with increasing methanol content, similar to results seen with other non-ionic polypeptides. At 92% methanol, all samples were

found to adopt essentially 100% α-helical conformations (Figure 4, Figure S6). These results show that although it is highly likely there is sulfoxide stereochemical disorder in these samples, it does not hinder the ability of **2**^{O}-**7**^{O} to adopt stable α-helical conformations, even for **2**^{O} and **3**^{O} where sulfoxides are closest to the backbones. Consequently, the differences in chain conformations observed for **2**^{O}-**7**^{O} in water must arise solely from differences in polypeptide solvation.

Here, the synthesis of five new non-ionic, water soluble sulfoxide containing homopolypeptides, as well as their incorporation into statistical and block copolymers was reported. Polypeptides **1**^{O}-**6**^{O} were found to adopt conformations in water that were dependent on distance of sulfoxides from the backbone and overall side-chain lengths. These homologs were found to be a useful model system for study of helix-coil transitions in water in the absence of contributions from charged groups or phase separation. The new amino acid sulfoxides may find use as hydrophilic guest residues with different conformational preferences in peptide sequences.[21] Also, since **2**^{O} has been shown to be cell and tissue compatible, degradable, and non-fouling, the new polypeptides here might find use in biomaterials applications. The ability to select non-ionic, water soluble polypeptides that are either disordered (e.g. **2**^{O}) or α-helical (e.g. **6**^{O}) in water has tremendous potential for controlling block copolymer assembly[22] and in protein therapeutic stabilization.[23]

ASSOCIATED CONTENT

Supporting Information. Supporting figures S1-S6, experimental procedures and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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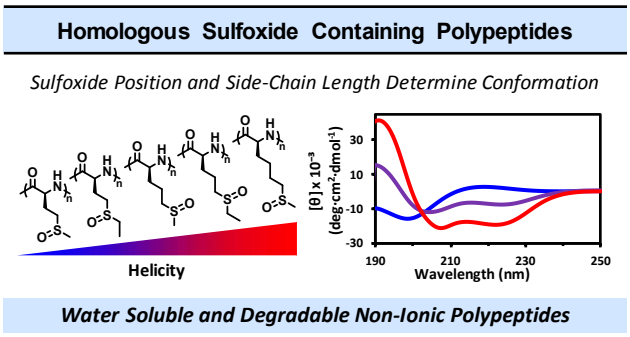
TOC Graphic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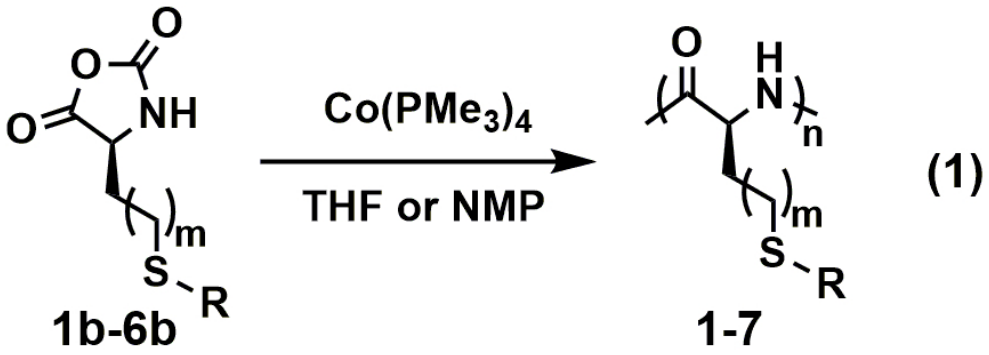

(1)

equation 1

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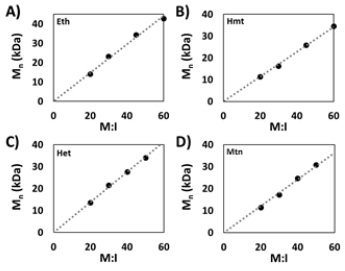


figure 1

254x190mm (96 x 96 DPI)

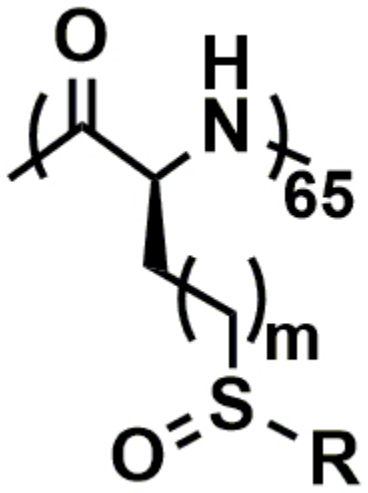


figure 2 graphic

16x22mm (300 x 300 DPI)

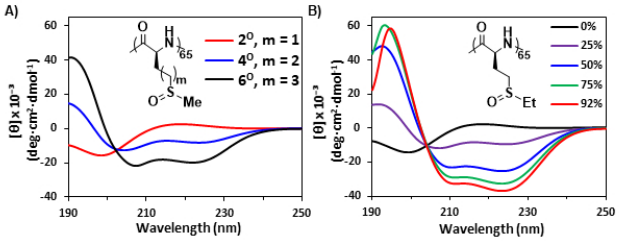


figure 3

254x190mm (96 x 96 DPI)

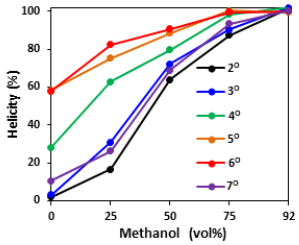
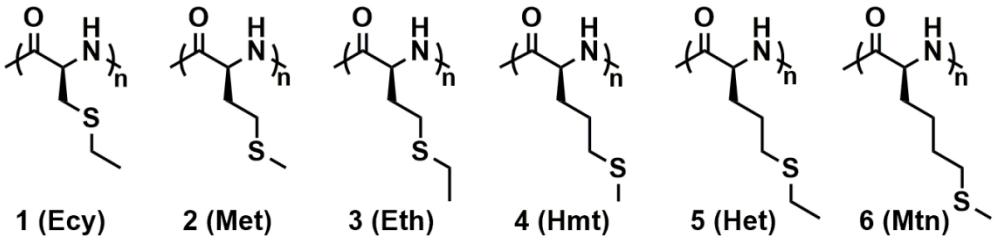


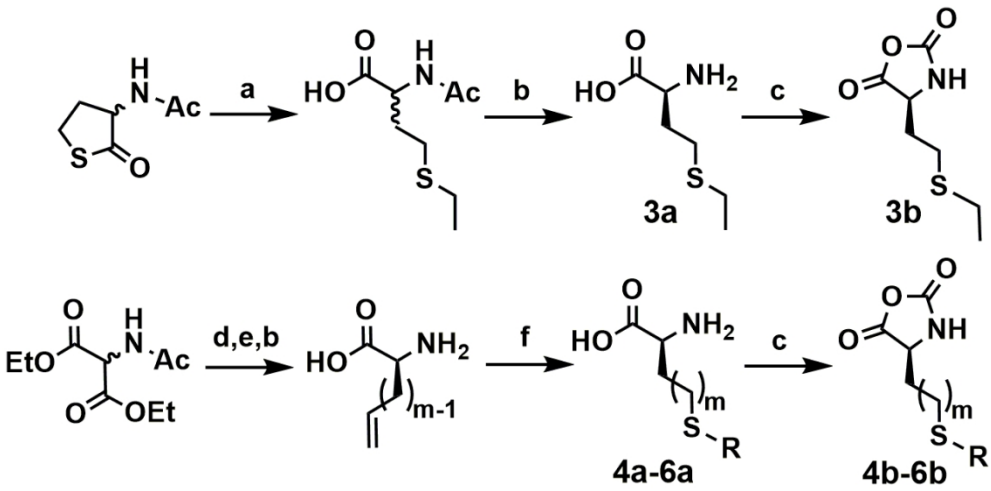
figure 4

254x190mm (96 x 96 DPI)



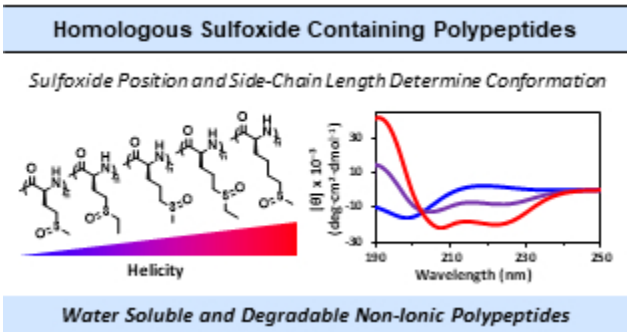
scheme 1

114x28mm (300 x 300 DPI)



scheme 2

110x55mm (300 x 300 DPI)



TOC graphic

82x44mm (96 x 96 DPI)